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57 Coronaviruses

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KEY POINTS

- Coronaviruses are widespread among mammals and birds, affecting many organ systems and causing a range of diseases.
- Human coronaviruses 229E and OC43 are major causes of the 'common cold'. These, as well as the newly discovered HCoV NL-63 and HKU1, can cause both upper respiratory tract infection and sometimes lead to lower respiratory tract infections in all age groups.
- SARS CoV emerged from bats, adapted in other small wild mammals (e.g. civet cats) and acquired efficient human transmission leading to a global outbreak of a novel disease. However, unusual features of its pathophysiology allowed public health measures to interrupt virus transmission in humans.
- No vaccines or antivirals are in routine clinical use for any HCoV.

Coronaviruses were discovered in the early 1930s when an acute respiratory infection of domesticated chickens was shown to be caused by a virus now known as avian infectious bronchitis virus (IBV). The first human coronaviruses (HCoV) were discovered in the 1960s. Research with human volunteers at the Common Cold Unit near Salisbury, UK, showed that colds could be induced by nasal washings that did not contain rhinoviruses. Subsequent in-vitro experiments, where nasal swabs from these volunteers were inoculated onto organ cultures of the respiratory tract, revealed the presence of enveloped viruses with the characteristic morphology of coronaviruses as previously described for IBV. The term *coronavirus* (Latin: corona, crown) was adopted for these agents, reflecting their characteristic fringed appearance in the electron microscope after negative staining. Coronaviruses are now recognized in a range of animal species causing respiratory, gastrointestinal, neurological and systemic diseases (Box 57.1). Until the emergence of SARS in 2003, only two, HCoV 229E and OC43, were recognized as human pathogens. Both were causes of the common cold, considered a mild and insignificant illness and thus not a high priority for intensive research. Following the recognition that SARS was caused by a novel coronavirus, two other new HCoVs, NL63 and HKU-1, were found in association with respiratory disease. The renewed interest in this group of viruses has led to the discovery of a plethora of other animal coronaviruses in diverse species and stimulated research on their capacity to cross species-barriers to infect new animal species.

TAXONOMY

Coronaviruses and *toroviruses* are two virus genera within the virus family Coronaviridae, order Nidovirales. Coronaviruses are well-established pathogens of humans and animals while the toroviruses are recognized as causes of animal diarrhoea. Toroviruses have also been found in human faeces but their aetiological role remains unclear.

Coronaviruses are classified into three groups, initially based on antigenic relationships of the spike (S), membrane (M) and nucleocapsid (N) proteins and now re-enforced by viral genetic phylogeny (Box 57.1). The HCoVs 229E and NL63 are group 1 coronaviruses, while OC43, HKU-1 and SARS coronaviruses are classified in group 2. Group 3 coronaviruses are found in avian species. Genetic recombination readily occurs between members of the same and of different coronavirus groups providing opportunity for increased genetic diversity.

Efforts to identify the animal reservoir of SARS coronavirus led to the discovery of diverse bat coronaviruses in both group 1 and 2 that are closely related phylogenetically to different mammalian coronaviruses. It has been proposed that bat coronaviruses

Box 57.1 Classification of coronaviruses

Group 1
- Human coronavirus (HCoV) 229E
- Human coronavirus NL63
- Porcine transmissible gastro-enteritis virus (TGEV)
- Canine coronavirus (CCoV)
- Feline infectious peritonitis virus (FIPV)
- Porcine epidemic diarrhoea virus (PEDV)
- Bat coronaviruses (e.g. 1A, HKU2)

Group 2
- Human coronavirus (HCoV) OC43
- Human coronavirus HKU1
- SARS coronavirus
- Rat coronavirus (RCoV)
- Rat sialodacro-adenitis virus (SDAV)
- Porcine haemagglutinating encephalomyelitis virus (HEV)
- Bovine coronavirus (BCoV)
- Mouse hepatitis virus (MHV)
- Bat coronaviruses (e.g. SARS-like coronavirus Rp3, HKU4, 229E like bat coronavirus)

Group 3
- Avian infectious bronchitis virus (IBV)
- Turkey coronavirus (TcoV)

may indeed have been the ancestors of many mammalian coronaviruses. It is noteworthy that recent studies on the comparative evolution of animal and human coronaviruses have led to the conclusion that HCoV 229E and OC43, the causes of the common cold which are now globally endemic in humans, crossed species from their animal reservoirs (bats and cattle, respectively) to humans within the last 200 years, illustrating the fact that coronaviruses continue to cross species barriers and cause novel diseases.

PROPERTIES

Morphology and structure

Coronaviruses are pleomorphic and enveloped, varying between 60–220 nm in diameter in negatively stained virus particles. Club-shaped surface projections or peplomers (composed of trimers of spike (S) protein) of approximately 20 nm in length are seen in all species, giving the particles their characteristic fringed appearance (Fig. 57.1). Some group 2 coronaviruses (OC43, bovine coronavirus) have an additional shorter haemagglutinin-esterase protein on the virus surface which forms a distinct inner fringe of short peplomers.

Coronaviruses have a non-segmented single-stranded positive-sense RNA genome of approximately 30 kb, making these the largest known RNA virus genomes. In the virion, viral RNA is complexed

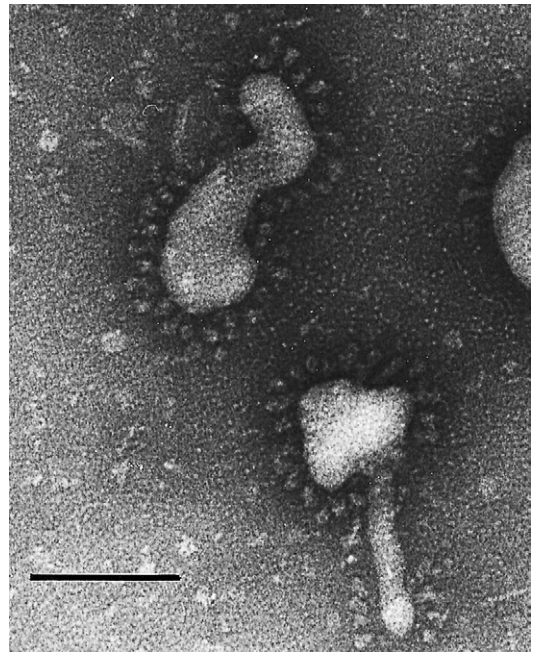

Fig. 57.1 Particles of HCoV serogroup 229E grown in human fibroblast cells and stained with 1.5% phosphotungstic acid. Bar = 100 nm.

with nucleoprotein (N) in an extended helical nucleocapsid 9–11 nm in diameter. This is enclosed within a lipid-bilayer membrane envelope in association with a transmembrane protein (M), which is the most abundant virus structural protein. The spike (S) glycoprotein, smaller amounts of a non glycosylated envelope (E) protein, and in some group 2 viruses, also the haemagglutinin-esterase (HE) protein, are also found on the virus envelope.

The S protein is the major inducer of neutralizing antibody, although when it is present, the haemagglutinin esterase protein is also a target for neutralizing antibody. Monoclonal antibodies raised against M protein can neutralize infectivity in the presence of complement. Antigenic variation is a feature of the S protein, whereas the N protein is relatively conserved.

REPLICATION

Coronaviruses attach to their glycoprotein receptors on host cells via their S (and when present, the HE) proteins. The tissue tropism of coronaviruses is mainly determined by the S1 part of the S protein and by the type and distribution of respective receptors on the cell surface. An illustrative example comes from veterinary virology. Transmissible gastroenteritis virus

(TGEV) and porcine respiratory coronavirus (PRCV) are common causes of disease in pigs, the former causing gastrointestinal disease and the latter being a cause of respiratory disease. It has been found that PRCV arose from TGEV through a deletion in part of the S protein that dramatically altered the tropism of the virus from the gastrointestinal to the respiratory tract. Group 1 coronaviruses 229E and NL63 bind to the metalloproteases, human aminopeptidase N and angiotensin converting enzyme 2 (ACE-2) respectively. Group 2 coronaviruses bind to 9-O-acetylated neuraminic acid molecules on the cell surface. SARS coronavirus also uses ACE-2 as the receptor for virus binding and entry. The receptors for OC43 and HKU-1 have not been yet identified. Viral entry is mediated by fusion of the viral envelope with the host cell membrane or by receptor mediated endocytosis. The fusion of the viral and cell membranes (either at the cell surface or within the endocytic vesicle) is mediated by the S2 portion of the virus spike protein which functions as a class 1 fusion protein.

Once the viral RNA is released into the cytoplasm, an RNA-dependent RNA polymerase translated from the plus-stranded viral genomic RNA makes a negative strand template from which it then synthesizes a series of 3′ co-terminal nested genomic mRNAs. The viruses replicate in the cytoplasm with a growth cycle of 10–12 h. Newly forming virions bud into the rough endoplasmic reticulum (where the M protein localizes) and accumulate into intracytoplasmic vesicles (Fig. 57.2). These newly formed virions are transported via the Golgi apparatus to the plasma membrane where they are released by exocytosis. Viral infection may result in cell lysis or fusion of adjacent cells may lead to the formation of syncytia.

PATHOGENESIS

Infection with the common-cold coronaviruses leads to loss of ciliary action (ciliostasis) and degenerative changes affecting the cilia of epithelial cells of the respiratory tract. Direct cell cytolysis is not prominent although this may also contribute to pathogenesis. The mechanisms of pathogenesis of HKU-1 and NL63 are not yet well studied. SARS CoV targets type 1 and type 2 alveolar epithelial cells of the lung and also differentiated bronchial epithelial cells. The desquamation of alveolar epithelial cells leads to hyaline membrane formation within the alveoli and diffuse alveolar damage, the histological hallmark of acute respiratory distress syndrome (ARDS). Patients with SARS have elevated levels of pro-inflammatory cytokines (IL-6, IL-12) and chemokines (IL-8, CCL-2, CXCL10) in the plasma but whether these mediators drive disease pathogenesis or are simply the consequence of the lung pathology remains unresolved. However, viral load in the upper respiratory tract peaks around days 7–10 of the disease and falls thereafter, while the lung pathology appears to progress through the second week of illness, suggesting that the lung pathology continues to be driven by mechanisms other than viral replication alone. The severity of SARS infection in humans increased with age, and interestingly, a similar phenomenon is also observed in SARS CoV infected mice and primates. The SARS CoV also infects the intestinal epithelium and virus is shed in the faeces. The diarrhoea associated with SARS infection may be related in part to direct infection of the intestinal tract.

A possible link between multiple sclerosis and coronaviruses has been investigated for some time. The genomes of Coronavirus 229E and OC43 have been detected in the brain tissue of patients with multiple sclerosis. However, these virus genomes are also detected in persons dying of non-neurological causes and thus the aetiological link between coronaviruses and neurologic disease in humans seems unclear. Some animal coronaviruses, such as variants of mouse hepatitis virus, can cause demyelinating CNS disease following experimental infection in the mouse.

TRANSMISSION

The primary route of transmission of human coronaviruses is via the respiratory tract. Experimental transmission of disease was demonstrated by the intra-nasal inoculation of adult human volunteers with 229E and OC43. These viruses have also caused outbreaks of nosocomial disease. Implementing contact and droplet precautions reduced its transmission in health care settings suggesting that respiratory droplets and direct or indirect contact was the major route of transmission. SARS CoV was found to retain infectivity on smooth surfaces for longer than some other human respiratory coronaviruses or other respiratory viruses suggesting the potential importance of fomites and indirect contact in its transmission. However, there was also evidence of small-particle aerosol (long-range) airborne transmission associated with aerosol generating procedures (e.g. use of nebulizers, intubation, high-flow oxygen therapy). SARS coronavirus was also excreted in faeces. Aerosolized faecal material from a faulty sewage system has been proposed as the mechanism of spread in one high-rise housing estate in Hong Kong where one index case led to many hundreds of secondary cases.

EPIDEMIOLOGY

Studies using virus detection or serology have shown that HCoV 229E, OC43 and NL63 occur worldwide. Although data on HKU1 is more limited, it too has a global distribution and has been found wherever it has been diligently sought. Initial infections occur early in life but re-infection continues to occur at all ages. There is no cross-protection between different types of coronavirus and immunity to the same virus type is also short lived with re-infection being documented within a few months. They have a winter-spring seasonality in temperate and sub-tropical climates. In contrast to viruses such as influenza or RSV which cause predictable annual outbreaks, the contribution of each HCoV may vary widely from year to year, for example 229E contributing as little as 1% to acute respiratory infections in the community in one year and up to 35% in the next. Furthermore, activity may be hetrogeneous in different geographic regions of the same country.

SARS coronavirus

The epidemiology of SARS CoV deserves special mention, because it highlights the emergence and control of a novel human infectious disease. SARS CoV emerged from a precursor virus which is endemic in insectivorous bats. The close proximity of different animal species (including bats) within large live game-animal markets which service the restaurant trade for exotic food in southern China allowed the bat SARS CoV-like precursor virus to adapt to other mammalian species (civet cats, raccoon dogs) and subsequently, to humans. Initial infections in late 2002 were asymptomatic and did not lead to onward transmission but the virus finally adapted to efficient human transmission leading to large outbreaks of disease in Guangdong Province, China, in February 2003. One infected patient from Guangdong travelled to Hong Kong and stayed one day at a hotel there leading to the infection of 15 other guests who travelled onwards to Toronto, Singapore, Hanoi and elsewhere, seeding chains of secondary transmission in different parts of the world. Within months, the outbreak had spread to 29 countries and regions causing over 8000 human cases and almost 800 deaths.

SARS was characterized by explosive outbreaks of disease in the community as well as in healthcare settings, 21% of all cases worldwide being nosocomially acquired. While many patients did not transmit infection at all, a few patients (so called 'super-spreading events') were responsible for large numbers of secondary cases. Such outbreaks appeared to be associated with a constellation of factors related to host, environment and circumstance and was not explained solely by host factors such as viral load in the patient's respiratory tract. The 'super-spreader' phenomenon has been described also in other infectious diseases.

By July 2003, determined and coordinated global public health measures had interrupted transmission in humans. SARS was unusual in two respects of its pathophysiology that allowed its transmission to be interrupted by public health measures. Unlike other respiratory viruses where the viral load in the upper respiratory tract and transmission is maximal early in the disease, in SARS, peak viral titres in the upper respiratory tract and maximal transmission typically occurred in the second week. This allowed early case-detection and isolation to interrupt community transmission. Furthermore, most infected persons manifested clinically overt disease, and thus, once symptomatically ill patients were detected and isolated, there was little asymptomatic infection in the community to sustain virus transmission. Other respiratory viral pathogens such as influenza are transmitted soon after, or even before, the manifestation of clinical symptoms and much of the infection remains mild or asymptomatic. Thus, while the spread of SARS was interruptible by public health measures, the influenza pandemic of 2009 was not.

New zoonotic infections of SARS emerged from the live game-animal markets in Guandong Province, China in December 2003 and January 2004. But these were caused by viruses poorly adapted to human transmission. Action to remove the potential animal sources of infection avoided a re-emergence of SARS. There were four other instances of human infection with human-adapted SARS CoV arising from laboratory accidents. In one of these instances, there was secondary transmission to contacts in the community, but prompt detection and case-isolation prevented a major outbreak.

CLINICAL FEATURES

229E and OC43 are associated with around 25% of common colds and are second only to rhinoviruses as the cause of this syndrome. Human volunteer studies have established that the incubation period is around 2 days with peak symptoms occurring at three to four days post infection. Subclinical or mild infections are common. The symptoms of nasal discharge, mild sore throat, sneezing, sometimes together with headache and general malaise lasts for 6–7 days. Fever and

cough are found in a minority of cases. Around 10% of children with otitis media have evidence of coronavirus infection. Coronaviruses have also been found in some patients with lower respiratory tract infections but as they may also be found in a proportion of asymptomatic controls, their aetiological role is difficult to establish. HCoV 229E, OC43, NL63 and HKU1 have all been identified in bronchoalveolar lavages in immunocompromised patients with lower respiratory tract disease suggesting that they contribute to severe respiratory illness in these patients. Serological studies have shown an association between coronavirus infections and exacerbations of respiratory symptoms in adults with underlying respiratory diseases or asthma. HCoV infections in the elderly with underlying respiratory disease may lead to lower respiratory tract disease although rarely severe enough to warrant hospitalization.

NL63 and HKU1 have been associated with a range of symptoms including fever, cough, rhinorrhoea, pharyngitis, bronchiolitis, pneumonia and febrile seizures. NL63 has also been strongly implicated as a cause of croup. Between 50–80% of patients with HKU1 infections had other underlying diseases.

SARS coronavirus

Although SARS CoV is not presently transmitting in the human population, the clinical features of SARS are instructive as an example of a severe viral respiratory disease. The incubation period of SARS was estimated to be 2–14 days. The disease presented as fever, myalgia, chills and a dry cough of acute onset leading to a rapidly progressing viral pneumonia. Upper respiratory symptoms of rhinorrhoea and sore throat were less common. Some patients had a watery diarrhoea. Ground glass opacities and focal consolidation predominantly involving the lung periphery and lower lobes was seen on radiographic examination. Some patients progressed to increasing tachypnoea, oxygen desaturation and respiratory distress syndrome. Moderate liver dysfunction and marked lymphopenia was seen. Central nervous system manifestations were reported but rare. The overall case fatality rate was 9.6%. The severity of disease increased with age and with the presence of underlying co-morbidities.

Gastrointestinal disease caused by coronaviruses and toroviruses

Coronavirus-like particles have been detected by electron microscopy in stool from diarrhoeal as well as healthy subjects and their role in diarrhoeal disease has remained controversial. A few human enteric coronaviruses (HECoV) have been successfully cultured in human embryonic intestinal organ culture. They appear to be endemic throughout the world, with a higher prevalence in developing countries. In western countries the prevalence is high in travellers from developing countries and in low socioeconomic groups, and is markedly higher in male homosexuals than in the normal population. There is strong circumstantial evidence that HECoV are spread by the enteric or faecal–oral route. The observed high prevalence among western male homosexuals may be explained by oral–anal–genital contact.

More recently, HKU1 has been detected in stool as well as the respiratory tract of patients with diarrhoeal syndromes by molecular methods and it is possible that this virus disseminates beyond the respiratory tract.

Toroviruses (a distinct genus within the family coronaviridae; see section on Taxonomy) have also been found in association with gastroenteritis in humans. Clinically these cases were less likely to manifest with vomiting and more likely to have a bloody diarrhoea and were more common in the immunocompromised.

LABORATORY DIAGNOSIS

Respiratory specimens are the specimens of choice but some coronaviruses (HKU1, SARS CoV, enteric coronaviruses) can also be detected in stool specimens. Prior to the emergence of SARS, coronaviruses were regarded as insignificant pathogens and routine laboratory diagnosis was not regarded as important. Furthermore, isolation of coronaviruses from clinical specimens is technically challenging, some of them requiring inoculation onto organ cultures of human embryonic trachea (e.g. OC43-like viruses) or special cell-lines (e.g. human embryonic lung fibroblasts, HUH7, LLC-MK2, Vero-E6) together with multiple sub-passages for their detection, procedures not readily amenable to routine diagnostic practice. The human hepatoma cell-line HUH7 has been recently used for primary isolation of OC43, 229E and HKU-1 viruses from clinical specimens and NL63 has been isolated in LLC-MK2 and Vero B4 cells. Some avian and mammalian (not human) coronaviruses can be cultivated readily in embryonated eggs. Some coronaviruses have the ability to haemagglutinate red blood cells, a property that has been used to detect their growth in cell cultures. Direct antigen detection of virus infected cells in clinical specimens has been shown to be feasible, but validated reagents are not widely available and the method is not frequently used.

Detection of viral RNA by RT-PCR is the widely used method in recent times. Specific primers for

detecting 229E, OC43, NL-63 and HKU have been reported. However the limited sequence data available on non-SARS coronaviruses needs to alert us to the possibility that PCR primers designed on the basis of currently available viral genetic data may not encompass the full genetic diversity of these viruses. There are also consensus coronavirus-specific primers that are broadly reactive with many human and animal coronavirus types and these have been used to detect novel coronaviruses (e.g. HKU1) but they are typically less sensitive than good type-specific primers.

Electron microscopy of negatively stained stool specimens is useful for the detection of enteric coronaviruses and toroviruses. The two types of viruses are similar in size and may be difficult to distinguish by electron microscopic morphology but toroviruses typically exhibit a doughnut-like or rod-like appearance unlike typical coronaviruses.

Complement fixation, ELISA assays, immunofluorescence or virus neutralization tests have been used for serological diagnosis and for sero-epidemiology of coronavirus infections.

Discovery of a new human pathogen, SARS coronavirus

SARS presented as a severe progressive 'atypical pneumonia' with no pathognomonic features except a propensity to lead to clusters of disease in close contacts including healthcare workers. Initial investigations of suspected cases did not find conclusive evidence of known respiratory pathogens. The WHO set up a worldwide network of virological laboratories investigating SARS cases which discussed their results in daily teleconferences. Approaches taken to identify a novel pathogen included virus isolation (including cell-lines not typically used to grow respiratory pathogens) and electron microscopy (EM) on respiratory specimens including lung tissue obtained at open-lung biopsy or autopsy. Immunological methods for virus detection require specific antibodies reactive with the virus and PCR or RT-PCR methods predicate knowledge of the viral genetic sequence upon which PCR primers are based, information and reagents not available in the context of the emergence of a novel pathogen. However, consensus primers targeting regions of the viral genome conserved across viral genera or families, low stringency PCR and PCR using random primers are feasible approaches to detect novel pathogens and were deployed in the hunt for the aetiological agent of SARS. The initial findings independently came from three laboratories within the WHO network isolating a cytopathic-effect causing agent in fetal rhesus kidney cell-lines or Vero-E6 cells. Thin section EM revealed that these cells were indeed infected with a virus (Fig. 57.2A) and immunofluoresence tests showed that this agent was not reactive with antibodies to previously known respiratory pathogens. EM of negatively strained preparations of ultracentrifuged deposits of infected cells showed particles that were compatible in size and morphology to coronaviruses. EM of lung-biopsy tissue also revealed virus-like particles of comparable size. PCR amplicons generated by random primer based RT-PCR assays on infected and non-infected cells were compared and those unique to virus infected cells were genetically sequenced. Some sequences were found to have homology to those of the coronavirus family. In immunofluorescent tests using virus infected cells, sera collected early in the course of illness from these patients (acute sera) failed to react whereas convalescent sera from patients with suspected-SARS gave a strong reaction (Fig. 57.2B), suggesting sero-conversion to the novel virus in patients with this novel disease. Control sera from an uninfected population had no antibody to this newly isolated virus. Taken together, these provided strong circumstantial evidence of an association between the coronavirus isolated in cell culture and SARS. The partial virus genetic sequence was then used to design specific RT-PCR assays and the virus infected cells were used as substrates for serological diagnosis in immunofluoresence tests and enzyme linked immunosorbent (ELISA) assays. Koch's postulates were fulfilled by infecting macaques with the isolated virus and reproducing a disease similar to SARS. A short while later, the full genome of the novel pathogen was elucidated, confirming thereby that the aetiological agent of SARS was indeed a novel pathogen within group 2 of the Coronaviridae.

This experience demonstrates the importance of 'classical' virological methods (cell culture, electron microscopy), which are 'catch-all' methods indispensible for detecting novel pathogens. Such methods should not be completely replaced by newer PCR based molecular diagnostics. The sharing of information in 'real-time' within the WHO laboratory network allowed rapid progress to be made in identifying the new pathogen, in establishing consensus, in validating reliable diagnostic tests to diagnose SARS and in disseminating credible information about the disease and its diagnosis.

CONTROL

Given the sheer number of 'common cold' episodes, their inconvenience and economic impact, prophylactic strategies that target coronaviruses and

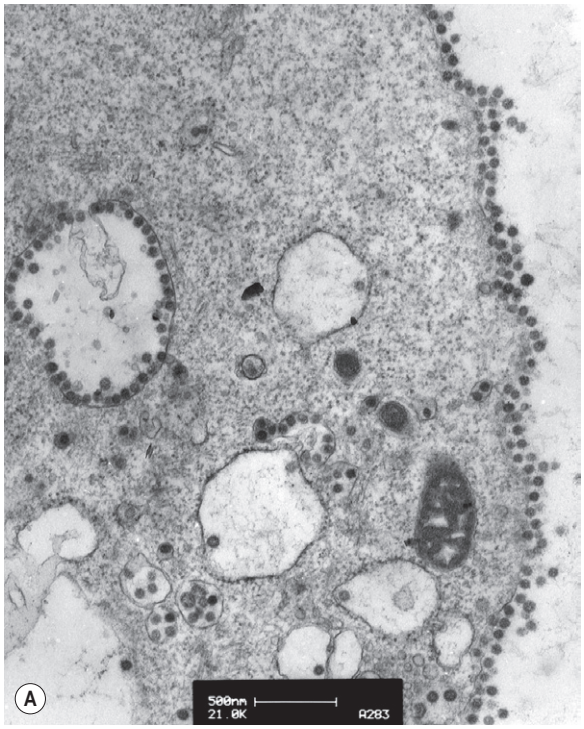

Fig. 57.2 (A) Thin section transmission electron microscopy of cells infected with SARS CoV showing virus particles in intracellular vesicles and on cell surface. Bar = 500 nm. (Courtesy of Dr JM Nicholls.) (B) Immunofluoresence reaction of a serum from a patient with SARS on SARS CoV infected cells. (Courtesy of Dr KH Chan.)

rhinoviruses (the two common aetiological agents of this syndrome) would be attractive. However, there are no validated antiviral drugs or vaccines to contain coronavirus infections so far.

TREATMENT

During the outbreak of SARS, given its severity and high mortality rates, a number of therapeutic options including ribavirin, interferon alpha, lopinavir/ritonavir, and nucleoside analogue protease inhibitor combination therapy were all tried. While there is evidence of activity in-vitro, these drugs were not evaluated in controlled clinical trials and their therapeutic benefit remains uncertain.

PREVENTION

Attempts to control transmissible gastroenteritis virus of pigs and feline coronavirus of cats through the use of vaccines have not been successful although vaccines for the avian disease infectious bronchitis virus has been modestly effective. The fact that natural infections with 229E or OC43 do not provide long-lasting immunity is instructive in this regard. Thus, so far, there is no vaccine for a HCoV that is in clinical use. The severity of SARS led to a concerted effort to develop vaccines for SARS CoV and range of vaccine strategies including inactivated whole virus vaccines, spike-subunit vaccines, DNA vaccines and vaccinia or parainfluenza virus type 3 vectored vaccines have all been tried in experimental animal models, with some providing evidence of efficacy. It has been established that antibody to the spike protein is the key correlate of protection in animal models. However, as there is perceived to be no imminent public health threat from SARS, few of these vaccines have been taken to human clinical trials. Passive immunotherapy using monoclonal antibodies that neutralize SARS CoV has also been developed and evaluated in experimental animal models of SARS.

RECOMMENDED READING

Bradburne AF, Bynoe ML, Tyrrell DA: Effects of a 'new' human respiratory virus in volunteers, *British Medical Journal* 3:767–769, 1967.

Falsey AR, Walsh EE, Hayden FG: Rhinoviruses and coronavirus infection-associated hospitalization among older adults, *Journal of Infectious Diseases* 185:1338–1341, 2002.

Gillim-Ross L, Subbarao K: Emerging respiratory viruses: challenges and vaccine strategies, *Clinical Microbiology Reviews* 19:614–636, 2006.

McIntosh K, Peiris JSM: Coronaviruses. In Richman DD, Whitley RJ, Hayden FG, editors: *Clinical Virology*, ed 3, Washington DC, 2009, ASM Press, pp 1155–1171.

Peiris JSM, Guan Y, Poon LLM: Severe acute respiratory syndrome (SARS). In Scheld WM, Hooper DC, Huges JM, editors: *Emerging Infections*, ed 7, Washington DC, 2007, ASM Press, pp 23–50.

van der Hoek L: Human coronaviruses: What do they cause? *Antiviral Therapy* 12:651–658, 2007.